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Structure-based β-secretase (BACE1) inhibitors

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25.1. Introduction

Alois Alzheimer identified first abnormal plaques in the brain of diseased people experiencing mental illness in 1906 [1, 2]. The illness is placing increasing burden on patients, as more people live long enough to become affected. It is clinically characterized by a progression from episodic memory problems to a slow global decline of cognitive function. At the final stage patients become bedridden and death occurs on average 9 years after diagnosis. The current standard of care includes treatment with acetylcholinesterase inhibitors to improve cognitive function. The NMDA (N-methyl-d-aspartate) antagonist memantine has also been shown to improve cognitive function in patients with moderate to severe Alzheimer's disease (AD) [3]. In addition, the common non-cognitive neuropsychiatric symptoms of AD such as mood disorder, agitation and psychosis often further require the application of medication. At this point, there is no approved treatment with a proven disease-modifying effect [1, 3].

Post-mortem analysis of human diseased brains provided the first clues to the mechanisms. It led to the description and the identification of the hallmark lesions of AD. The abnormal plaques are called β-amyloid plaques and neurofibrillary tangles. AD is characterised neuropathologically by the presence of amyloid β-peptide (Aβ) - containing plaques and neurofibrillary tangles composed of abnormal τ-protein. Some forms of Aβ are produced from the amyloid precursor protein (APP, Fig. 1); however, the Aβ42 (10% of all Aβ produced) appears to be the major pathogenic form and the most important component of amyloid plaques. The amyloid hypothesis of AD suggests that Aβ accumulation is the cause of the disease. APP is processed through the major α- and the minor β-secretase pathways that result in proteolytic fragments, which are further processed by γ-secretase. On the contrary to the nonpathogenic products of α-secretase, the β-secretase pathway produces pathogenic Aβ

peptides. BACE1 (β-secretase 1) was demonstrated to be the rate-limiting enzyme activity in the production of Aβ, suggesting a potentially beneficial effect of β-secretase inhibitors in AD [2, 4-7].

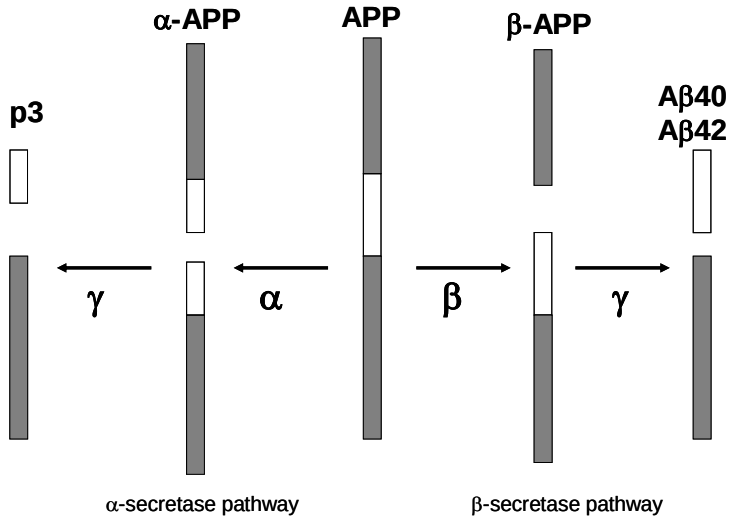

Figure 1. Amyloid precursor protein cleavage by proteases.

Although aspartic proteases [8] form the smallest protease class with about 15 members in the human genome, this class proved to be one of the richest pool for pharmaceutical research. Despite numerous attempts only HIV protease and renin inhibitors were brought to the market. Nevertheless, several inhibitors targeting renin, β-secretase (BACE1) and γ-secretase are in clinical or pre-clinical phase and some others are discussed as potential drug targets. Here we summarise the most important developments on one of the most popular aspartic protease target BACE1 [4,5 9].

25.2. BACE1: structure, dynamics and function

The number of the deposited BACE1 crystal structures in protein data bank reached 200 at the end of 2012. They have a general fold with three topologically distinct regions, an N-terminal, a C-terminal domain, and an intermediate domain consisting of six anti-parallel β-sheets. A

so-called flap loop extends over the active site that occurs in two states, an open or closed state with several intermediate positions between them. Residues 9-14 compose the second characteristic loop identified as the 10s loop [10, 11].

Although the catalytic site is conserved, BACE1 exhibits inherent flexibility. Apart from side-chain movements, the flap and the 10s loop show significant mobility. To capture these flap and 10s loop motions long molecular dynamic simulations are required. Several simulations have been run to study the loop motions and characterise the dynamic nature of BACE1 function [10, 12, 13, 14]. These studies also shed light on the hydrogen bonding network between conserved water molecules and the enzyme and also the protonation states of the catalytic aspartic acid residues. According to them there are two conserved water molecule within the active site one forming hydrogen bond with Ser35 and Asp32 while the other accommodates in the close proximity of Tyr71. Molecular dynamic simulations also supported that hydrogen bonding network exists only if Asp32 is neutral and Asp228 is deprotonated in the presence of OM99-2 ligand [12, 13, 15].

N- and C-terminal domain contributes one catalytic aspartic acid residue to the active site (Fig. 2), where the peptide bond cleavage occurs by a general acid-base catalytic mechanism. One of the aspartic residues is protonated and the other acts as a general base activating a water molecule which attacks the carbonyl carbon of the scissile amide, resulting in a tetrahedral geminal diol intermediate. To determine the protonation states of the catalytic dyad (Asp32/Asp228) various studies have been carried out ranging from experimental to theoretical calculations [12, 15, 16, 17]. We investigated protonation states of catalytic aspartates by electrostatic calculations that revealed the inner oxygen of Asp32 is protonated rather than Asp228. It was further supported by a comparative virtual screening study of ours [7]. A recent study of Yu et al. supported our results by quantum chemical calculations

indicating that the preferred state of aspartic acids is monoprotonated in the presence of the ligand [17]. Their proposal is in agreement with the molecular dynamics study on BACE1 in the presence of OM99-2.

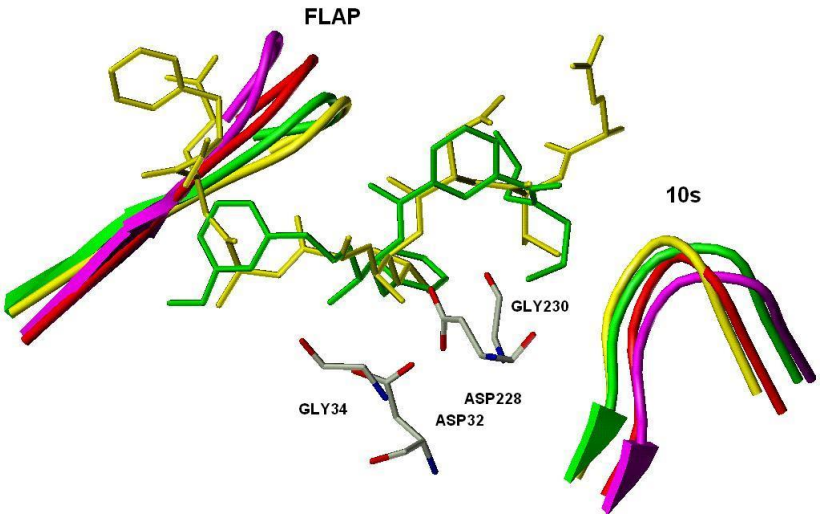

Figure 2. Schematic representation of BACE1 active site

25.3. Early attempts to discover BACE1 inhibitors

The knowledge gained in the development of HIV protease and early renin inhibitors accelerated the design of BACE1 inhibitors, which was further upheld by X-ray crystallography.

HIV protease inhibitors (e.g. amrenavir, indinavir, lopinavir) are all peptidomimetic inhibitors designed by a general approach of truncating a substrate peptide and replacing the scissile amide bond with a non-cleavable transition state isostere. Statine, homostatine and norstatine are the classic isosteres (Fig. 3) and have been applied thoroughly in the design of BACE1 inhibitors. The most prominent ones are OM99-2 and OM00-3 discovered by Tang and Ghosh

[11, 18, 19]. Hydroxyethylamine isosteres (Fig. 3) have one less amide bond than the statine isosteres and are extensively applied in BACE1 inhibitor design [20, 21, 22, 23, 24, 25].

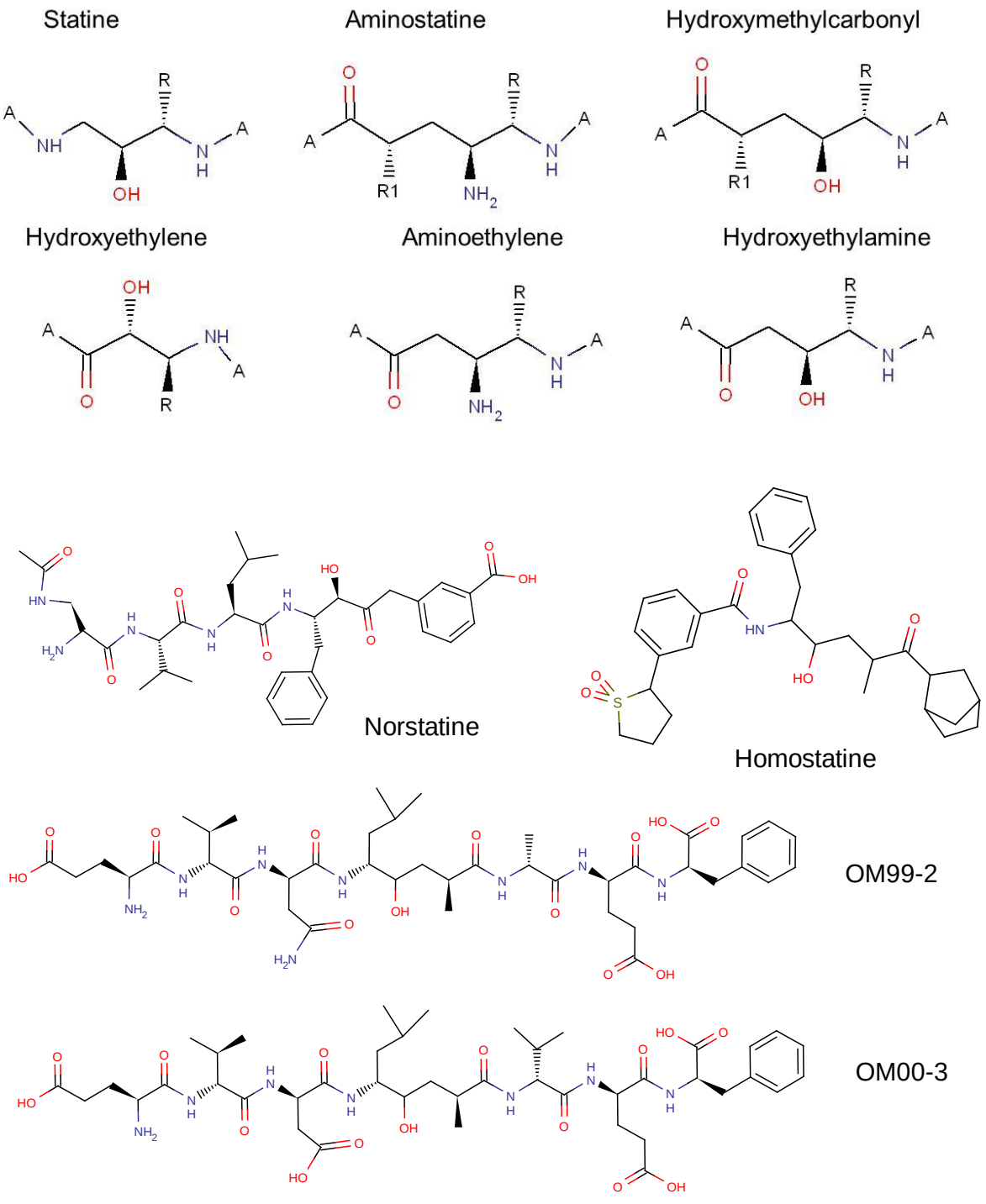

Figure 3. Transition state analogues that mimic the tetrahedral intermediate, A represents any atom or group.

Classic non-peptidomimetic aspartyl protease inhibitors were discovered by traditional, low-throughput screening methods. Furthermore, BACE1 inhibitors with adequate metabolic stability, oral bioavailability and blood-brain barrier penetration were pursued extensively by high-throughput screening methods. Acylguanidines were discovered by HTS FRET assay [26]. Although the primary hit showed only modest enzyme activity, the structure-based design of follow-up compounds resulted in potent compounds. Other relevant groups of BACE1 inhibitors are iminohydantoins [26], 2-aminothiazoles [26], tetronic and tetramic acids [26, 27].

Since the discovery of BACE1 in 1999 tremendous progress has been made but many hurdles remained before their clinical trials.

25.4. Structure - based design of BACE1 inhibitors

25.4.1. Fragment-based approaches

So far various kinds of approaches have been applied to discover BACE1 inhibitors, however most of them aimed at the design of peptidomimetic inhibitors. A potential problem with them is they tend to be large and possess multiple hydrogen bonding donors. Therefore these often cannot get through the blood-brain barrier. Consequently there is a considerable interest to discovery less peptide-like and small BACE1 inhibitors [3, 28, 29].

Fragment-based lead generation has recently emerged as an alternative to traditional high-throughput screening to identify initial chemistry starting points for drug discovery programs. In comparison to HTS screening libraries, the screening sets for fragment-based lead generation tend to contain orders of magnitude fewer compounds, and the compounds themselves are less structurally complex, have lower molecular weight and higher solubility

[28]. Here we only summarise the advent of fragment-based lead generation, however public literature discusses more [30-35].

Murray et al. performed a virtual screen on the Astex corporate library to identify low molecular weight fragments [34]. The GOLD docking algorithm with GoldScore and ChemScore was applied. The top scoring fragments then were soaked into the X-ray structure of BACE1. The study demonstrates that fragments can exhibit measurable IC_{50} (half maximal inhibitory concentration) in the range of 1-2 mM and scoring functions could reliably filter fragments (Fig. 4). Fragments typically would have low binding affinities in the range of a few mM - 100 μM compared to virtual screening hits or other structure-based approaches that are usually in the range lower μM.

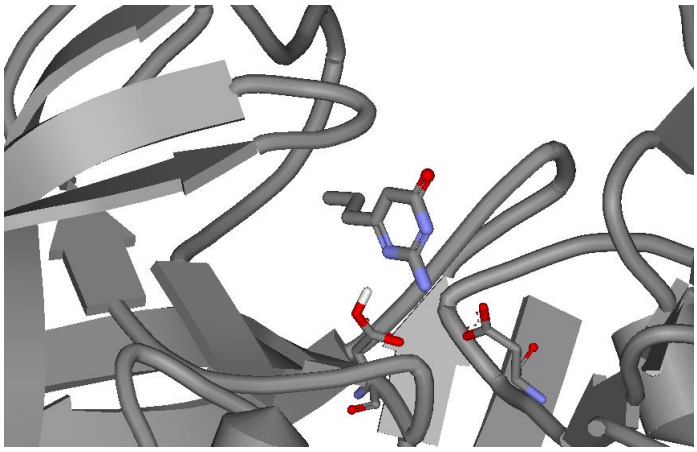

Figure 4. Binding mode of a fragment found by fragment-based virtual screening (PDB code: 3HVG)

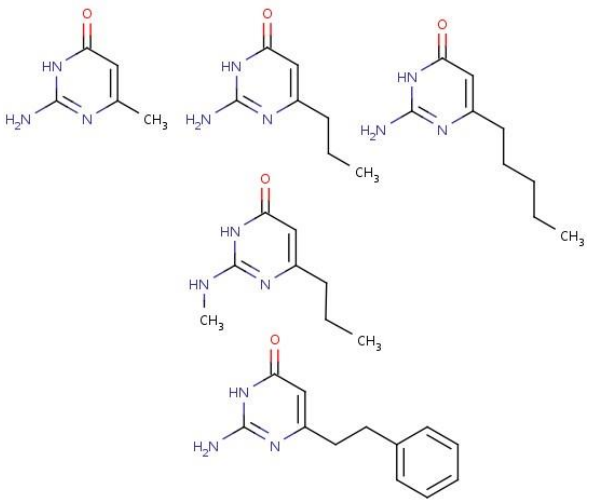

Figure 5. Active isocystine analogues

A fragment-based lead generation approach was shown to find novel small-molecule inhibitors of BACE1. The study stream consisted of 1D NMR for the primary fragment screening, and both NMR and BIAcore (label-free interaction analysis) in the subsequent analoguing phase to characterise FRIT (fragment hit) series and map some initial structure-activity relationships. Crystallography efforts were made for the established binders and optimised using biophysical methods, considering their different throughputs versus the richness of the delivered information. Problems arising due to the inability to grow crystals at the biologically relevant pH at which the screen was run were overcome by using endothiapepsin as a surrogate aspartyl protease. A series of 6-substituted isocytosines (Fig. 5) as a novel scaffold for BACE1 inhibitors was identified, and these were later optimised to a lead series of nanomolar inhibitors [35]. With this result in hand, a search was performed in the corporate collection for compounds bearing the isocytosine substructure. Several such compounds were identified, but they were either inappropriately substituted such that they could not bind to BACE1 or were not large enough to afford sufficient potency to lit up in an

HTS screen. This underpins the claim of fragment-based lead generation that working with fragment-sized molecules allows for a more efficient sampling of compound space [35].

Godemann et al. discovered novel non-peptidic inhibitors by functional fragment-based screening [36]. A diverse library of 20.000 compounds was initially screened against BACE1. The primary screen identified various low affinity BACE1 inhibitors and one of them was further characterised in biochemical assays. Competitive inhibition observed for this compound prompted the authors exploring its binding mode by X-ray crystallography and confirm the structure-activity relationship.

Huang et al. combined *in silico* screening consisting of fragment-based docking, ligand conformational search by a genetic algorithm and evaluation of free energy of binding to identify low molecular weight inhibitors of BACE1. More than 300.000 small molecules were screened (Fig. 6) and about 15.000 were prioritised by according to a linear interaction energy model. Eighty-eight compounds were tested *in vitro* and 10 of them containing a triazine scaffold showed an IC_{50} value better than 100 μM [37].

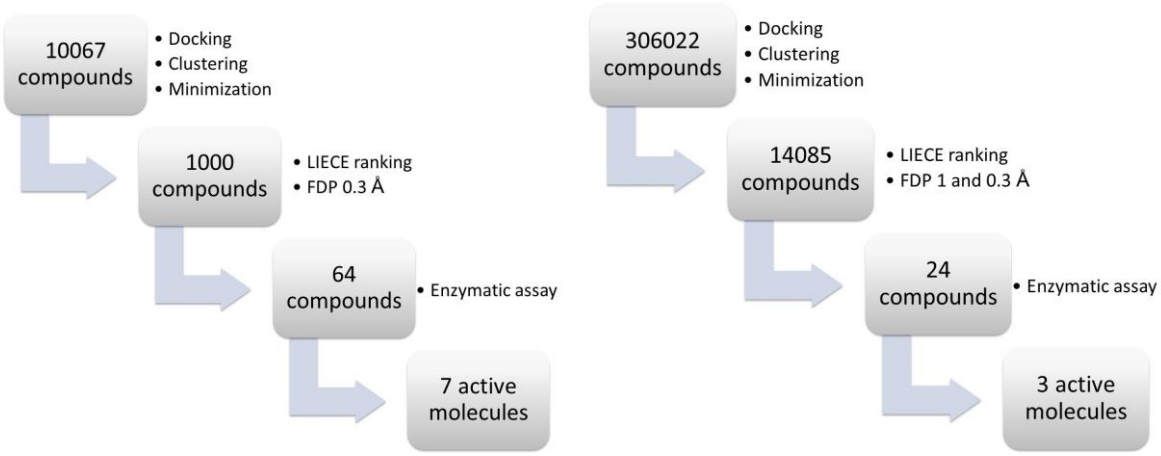

Figure 6. Schematic picture of the two in silico screening approaches. FDP stays for finite-difference Poisson calculations.

A fragment-based X-ray screen of Pfizer's proprietary fragment collection has resulted in the identification of a novel spiropyrrolidine BACE1 inhibitor. Although the primer hits showed only weak inhibitory activity, further optimization of the lead compound, relying heavily on structure-based drug design and computational prediction of physiochemical properties, lead to a 1000-fold improvement (Fig. 7) in potency while maintaining adequate ADME properties [38].

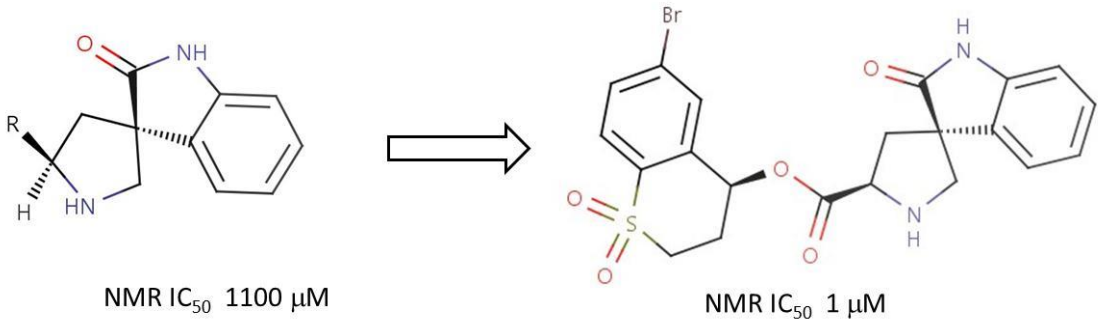

Figure 7.

Wyss et al discovered an isothiourea fragment through target-based NMR screening. The determination of its X-ray crystal structure in complex with BACE1 revealed an extensive H-bond network with the two active site aspartate residues. This detailed 3D structural information then enabled the design and validation of novel, chemically stable and accessible heterocyclic acylguanidines as cores. Structure-assisted fragment hit-to-lead optimisation yielded iminoheterocyclic BACE1 inhibitors that possess desirable molecular properties as potential therapeutic agents [39].

25.4.2. Pharmacophore-based approaches

Pharmacophore is an arrangement of steric and electrostatic features in the three dimensional space that are crucial for the biological action. Pharmacophore models usually consist of a

number of pharmacophore points including a group of atoms, or features such as hydrogen bond donors and acceptors, charged groups, hydrophobic centres and corresponding geometric constraints. Pharmacophore features can be derived from both the structure of the target protein or from the collection of known ligands. Structure-based pharmacophores are typically explored by analyzing binding site interactions formed between ligands and protein atoms within the active site of the target. Hydrogen bonding features, as well as electrostatic and hydrophobic interactions are utilised to derive the most important interactions within the active site.

A Vertex patent application [40] claims the following pharmacophore for BACE1 inhibitors: (1) hydrogen bonding interactions with one or both of the catalytic aspartic acids (Asp32, Asp228), (2) hydrogen bond donor interactions with Gly34 and/or Gly230, (3) occupation of the 'flap pocket' S1, S2 or S20 pockets (Fig. 2), and (4) stacking interactions with Tyr71, Phe108, and/or Trp76 (Fig. 8). We incorporated these pharmacophoric elements in our virtual screening study [7, 41]. In particular, the hydrogen-bonding interactions described by (1) and (2) above were incorporated, leading to an enrichment factor of 41 in the top 1% of compounds retrieved. A recent article [42] on ensemble-docking using pharmacophore points also derived a 9-point pharmacophore model, which roughly fits 5 of the Vertex pharmacophore points while also including 4 unique ''accessory points of interaction.'' They propose that these correspond to common geometric and electronic features essential for enzyme inhibition that can aid the rational design of new BACE1 selective inhibitors [15].

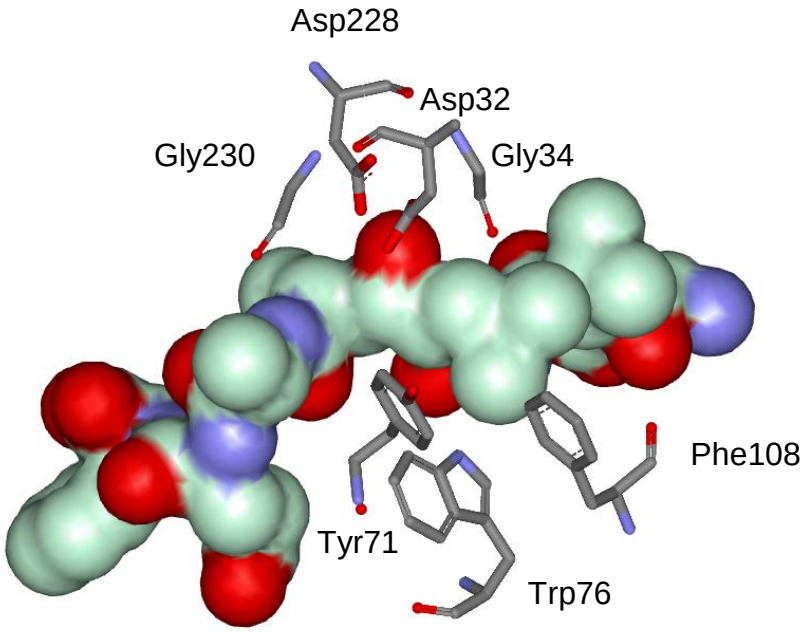

Figure 8. Vertex pharmacophore (sticks) with a peptidomimetic ligand (CPK)

25.4.3. High-throughput docking

Several papers have reported the design of BACE1 inhibitors guided by the crystal structure of the catalytic domain. Here we mention some of the successful case studies.

In a previous study of ours we performed a comparative virtual screening study on BACE1 [7, 41]. For virtual screening FlexX and FlexX-Pharm was applied on an apo (1SGZ) and a peptidomimetic ligand bound (1FKN) crystal structure. Our goal was to explore the impact of Vertex pharmacophore constraints on docking performance and to check the ability of algorithms to handle various conformations of the same protein. The influence of protonation states of Asp32 and Asp228 on docking experiments was also studied. The main point in our scoring scheme was to handle pose prediction and ranking separately. Five scoring functions (FlexX, DOCK, ChemScore, GoldScore, and PMF) were used for both pose extraction and ranking, resulting in 25 different scoring combinations in total. For docking, two algorithms

(FlexX and FlexX-Pharm) were applied, and two protonation states (calculated and default) were considered, and the 25 different scoring combinations resulted in 100 enrichment factors for each protein conformation calculated at 1% of the ranked database, which contained 9950 inactive and 50 active compounds. Docking with FlexX and setting default protonation states (Asp32 and Asp228 are both deprotonated), the 1SGZ crystal structure seemed to provide preferable enrichment factors over 1FKN. Introduction of Vertex pharmacophore constraints by FlexX-Pharm improved enrichment factors for both structures. The effects of protonation states were investigated in an enrichment study using both FlexX and FlexX-Pharm algorithms. Protonation states were determined by ZAP-pKa [7]. Docking into 1SGZ by FlexX at calculated protonation (Asp32 is protonated and Asp228 is deprotonated) states yielded similar enrichment to that obtained by FlexX-Pharm with default protonation. In case of 1FKN we observed similar trends; consideration of calculated protonation states has an effect comparable to that of pharmacophore constraints. In summary, we obtained comparable enrichment factors for two protein conformations by putting emphasis on protonation and pharmacophore constraints. FlexX often finds solutions on the outer surface of the active site and therefore could not provide sufficiently accurate poses. In fact, these solutions reduced our enrichment. Pharmacophore constraints can solve this problem and could improve the enrichment significantly. Protonation states of catalytic residues played an important role in positioning and scoring ligands. 1FKN is a ligand-bound conformation, and therefore, ligands could find proper poses easier than in the open structure (1SGZ). In 1SGZ, however, pharmacophore constraints and proper protonation could recover the results of 1FKN given by FlexX-Pharm (default protonation) or FlexX (calculated protonation). All cases DOCK score provided the best results.

A study of Xu et al. describes an efficient approach by integrating virtual screening with bioassay technology for finding small organic inhibitors targeting BACE1. Fifteen hits with inhibitory potencies ranging from 2.8 to 118 μM (IC_{50}) against β-secretase were successfully identified. Compound 12 with IC_{50} of 2.8 μM is the most potent hit against BACE1. Docking simulation from GOLD 3.0 suggests putative binding mode of 12 in BACE1 and potential key pharmacophore groups for further designing of non-peptide compounds as more powerful inhibitors against BACE1 [43].

Induced-fit docking of the highly flexible BACE1 inhibitors into the flexible BACE1 active site [44] was applied by Moitessier et al. Encoding the enzyme/inhibitor complex as a chromosome and using a 2-point crossover operation allowed the rapid docking and scoring of inhibitors in multiple conformational states of the enzyme. For a training set of 50 known flexible inhibitors, their protocol for induced-fit docking yielded an RMSD of 1.19 kcal/mol using a new force-field based scoring function, RankScore, which incorporates terms for desolvation, protein entropy loss, and complex solvation. In a head-to-head comparison using their flexible docking protocol, RankScore was superior to LigScore2 and PLP2.

Another flexible docking study published by us compared the performance of FlexE with that of FlexX and FlexX-Pharm by carrying out virtual screening experiments on BACE1. FlexE considers multiple side-chain conformations and loop movements by either employing multiple protein structures simultaneously or by combining multiple protein structures via a united protein description. Unfortunately, the results demonstrated that, although moderate loop motions (displacement of 2.5 Å) were handled well by FlexE, the enrichments in virtual screens were lower than those obtained by individual FlexX runs over multiple structures [41].

Kacker et al combined quantum mechanical calculations, molecular dynamics, and conformational ensemble virtual ligand screening addressing multiple protonation states and the conformational flexibility of the active site simultaneously. A methodical investigation of 146 enzyme–inhibitor complexes was performed resulting seven clusters. A representative of each cluster was undertaken DFT quantum mechanical calculations determining the dyad (Asp32, Asp228) protonation states, following molecular dynamic calculations to study these effects on the active site flexibility. Moreover, self-docking studies were performed to demonstrate the relevance of the aforementioned factors proving the importance of computational methods in BACE1 inhibitors discovery campaigns [15].

25.4.4. Attempts to predict BACE1 binding affinity

One main goal of the modelling studies is to predict the binding free energy between BACE1 and a ligand. Many approaches have been proposed to handle this problem such as empirical models for scoring functions, theoretical estimations of the free energy of change, models based on linear relationship between binding and computed interaction energy terms incorporating solvation explicitly or implicitly in many force-fields [4, 5, 45].

Tounge and Reynolds reported calculations of the binding affinity of BACE1 inhibitors [46]. Using the Linear Interaction Energy (LIE) method developed by Åqvist [47] and a set of 11 peptide inhibitors [48], they developed a binding affinity model that resulted in an RMSD between predicted and observed binding energies of 1.1 kcal/mol. They demonstrated that the model is not significantly dependent on the method used for calculating LIE terms, and moreover the entire BACE1 enzyme need not be included in the calculation. An improved LIE model was later reported [49], which applied a slightly different functional form of the computed interaction energies. This model was used to study subsite specificity for the P2

through P20 positions published by Turner et al. [50] and to evaluate a small number of C-terminal analogues reported by Hom et al. [51]. The LIE model was generally able to correctly reproduce the activity trends. This work was later extended by examining the van der Waals, coulumbic, and continuum solvation contributions to the computed binding affinities and by varying the protonation state of the enzyme and ligands [52]. The computed binding affinities were found to be relatively insensitive to the protonation state of the protein when the ligands were neutral; however, inclusion of charged ligands led to large shifts in all of the terms. Based on these results, it was recommended that the protein be judiciously charged and that all ligands (even potentially charged ones) be treated as neutral. The success of the LIE studies validated the use of a computational approach to estimate the binding affinity of BACE1 inhibitors. However, due to the cost ineffective calculations, they may not be practical for high-throughput methods. The LIE method has also been combined with energy minimisation and finite difference Poisson calculation of electrostatic solvation, i.e., Linear Interaction Energy Continuum Electrostatics (LIECE), to speed up the computations [46]. The results of a study of 13 peptidomimetic BACE1 inhibitors [56] and 29 HIV1 protease inhibitors indicate comparable accuracy (RMSD of about 1.0 kcal/mol) to LIE. However, the authors estimate that this approach still requires about 5 min per compound.

A method employing simple interaction energy calculations between a ligand and the BACE1 active site used by Holloway et al. improved accuracy [52]. In this study several scoring functions and MMFFs94 force field were applied to predict the binding free energy. Better or similar performance was observed than using computationally expensive methods for given series of ligands, these appear to be simple and rapid scoring functions for ranking virtual hits of high-throughput docking studies.

A much faster approach that has been applied for predicting the binding affinity of BACE1 inhibitors is calculation of the enzyme-ligand interaction energy using the MMFF94 force field [53]. Using the same peptidomimetic inhibitor training set [48] as the earlier LIE and LIECE studies, Holloway et al. [52] observed a high degree of correlation (r^2=0.85) between the computed interaction energy in a fixed enzyme active site and the observed K_i. Employing the equation of the fitted line to predict activity, the RMSD between predicted and observed binding energy for this series of inhibitors was 0.79 kcal/mol, slightly better than the more computationally intensive LIE and LIECE methods. This was reproducible for a second set of HEA-containing inhibitors (r^2=0.82). One might argue that in all of these studies the favourable RMSD is simply a result of the congeneric nature of the compounds in the test series. However, in order to capture the binding energy of more diverse molecules with varying S3 substituents, the interaction energy calculations using the MMFF94 force field have been extended by incorporating multiple fixed active sites [53]. In this study, 19 tertiary carbinamine inhibitors were examined in four different BACE1 X-ray structures that sampled 10s loop-up and -down conformations [54]. Since the inhibitors varied in the substituents accessing the S2 and S3 pockets of BACE1, but did not differ in the bioisostere or P1 group, only conformational flexibility of the P2/P3 portion of the molecule was explored. On average, 10 conformations per molecule were scored in the four BACE1 active sites and the lowest interaction energy was plotted against the experimentally determined K_i from the BACE1 enzyme assay. Considering energetically accessible conformations only these authors obtained significant correlation with an r^2 of 0.89. This study led to the theory that 10s loop motion depends on induced fit to the ligand, e.g., small non-polar P3 substituents appear to induce a 10s loop-down conformation in which S10 forms an H-bond with Thr232 to close the pocket. Conversely, large P3 substituents with an H-bond acceptor can replace the S10

interaction with Thr232 and fill the large hydrophobic pocket opened by a 10s loop-up conformation (Fig. 2). An advantage of having a 10s loop-down conformation is the overall reduction in molecular weight of the inhibitor with the potential for improved pharmacokinetic properties and increased brain penetration. Perhaps even faster scoring can be achieved by the development of 3D-QSAR models [55]. Thirty-two statine-based peptidomimetic BACE1 inhibitors [46] were docked into the BACE1 active site and their superposed bound conformations employed to derive CoMFA and CoMSIA models with q^2 values of 0.582 and 0.622, respectively. Both models recapitulated the key features of the BACE1 active site and were validated by predicting the activity of 4 of the 32 inhibitors that were split from the training set to serve as a test panel. It is unclear how broadly predictive these models would be, e.g., for a series of structurally distinct BACE1 inhibitors. A recent report [56] combines several of the scoring techniques described above into a multi-filter screening approach that begins with 2D substructure screening, proceeds through coarse docking and standard docking, and is followed by binding free-energy calculations and partial interaction energy analyses using MM/PBSA and protein alanine scanning. This in silico multi-filter screening retrieved all known inhibitors from the compound database investigated, suggesting that other compounds identified as inhibitors by this computerized screening process are potential β-secretase inhibitors [57].

Liu and co-workers performed a comparative binding energy analysis using (COMBINE). to explore the mechanism of inhibition. They used 46 X-ray crystallographic BACE1 inhibitor complexes to derive quantitative structure-activity relationship (QSAR) models. The inhibitors were aligned by superimposing the X-ray complexes. gCOMBINE software was used to perform the binding energy analysis on these 46 minimised complexes. The major advantage of the COMBINE analysis is that it can quantitatively extract key residues involved

in binding and identify the nature of the interactions between the ligand and receptor [58]. Considering the contributions of the protein residues to the electrostatic and van der Waals intermolecular interaction energies, two predictive and robust COMBINE models were developed: (i) the 3-PC distance-dependent dielectric constant model with a q^2 value of 0.74 and an SDEC value of 0.521; and (ii) the 5-PC sigmoid electrostatic model with a q^2 value of 0.79 and an SDEC value of 0.41.

25.4.5. Addressing BACE1 selectivity

Cathepsin D (Cat D) and renin are the related aspartyl-proteases to BACE1 and also can be found in the brain. Therefore BACE1 selectivity is important since the target enzyme resides in the brain, where closely related ubiquitous enzymes Cat D and renin can be found. BACE1 inhibitors with poor selectivity against these enzymes are likely to cause undesired side effects. To address this concern, the X-ray structures of renin, Cat D, and BACE1 were compared and significant differences in the length and sequence of the loop defining the S10/S30 pocket were noticed. The BACE1 loop is truncated (like the fungal aspartyl proteases) and primarily hydrophilic, whereas the renin and Cat D loops are extended and primarily hydrophobic. In addition, Arg235 (numbering based on the mature form), which resides between the P10 and P2 regions, is unique to BACE1 (Val233 in Cat D and Ser222 in renin) and may contribute to the preference for a P10 Asp in the BACE1 substrate APP. Based on this analysis, it seemed reasonable that the selectivity of a BACE1 inhibitor could be greatly enhanced by increasing the polarity of the side chain at the P10 position [52].

Selectivity over BACE2, a homologous isoenzyme is also important. Sequence differences of these isoenzymes include a Lys residue on the FLAP region in BACE2 rather than a Pro modifying its mobility. Although selectivity over renin, Cat D, Cat E, pepsin A is important in

the development of a BACE1 inhibitor there is no consensus on the need for a BACE1/BACE2 selective inhibitor [59, 60, 61].

25.5. Conclusions

Rich structural information that is publicly available accelerated the structure based support of BACE1 inhibitor design. Not only the experimental results added real value to the design but state-of-the-art methods that had been applied opened new routes for the design. Protein flexibility handled via induced-fit docking, calculated protonation states within the active site by molecular dynamics, quantum chemical or semi-empirical methods are typical examples of computational approaches that contributed to the discovery of BACE1 inhibitors.

Although computational approaches are relatively fast and inexpensive these are still not as reliable as experimental methods. The work of Murray et al. however clearly demonstrated that combining experimental and computational approaches can definitely lead to the discovery of novel compounds.

Despite the effort put in the quest for BACE1 drug discovery there are not as many studies as might be expected for a pharmaceutically important target of the aspartyl-protease family. Certainly with the first BACE1 inhibitor just being tested in phase one has remained the space wide open for further pharmaceutical research.

25.6. References

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